

## **PAN-LEUCOGATING (PLG) PROTOCOL .**

### **Specimen Collection:**

- 1). Collect blood (at least 3ml), in EDTA tubes.
- 2). Ship at ambient temperature
- 3). Run CBC prior, as the WBC is required for PLG.
- 4). Run each specimen on FACSCount, the 'gold standard' using 50ul of whole blood  
Per antibody tube, for each pair.

### **Sample Preparation:**

- 1). Label two (5ml) Falcon tubes CD4, CD8 respectively, for each specimen to be run and assign to each pair, the patient Id number.
- 2). To each labeled tube, add 5ul CD45 + 5ul CD4 or CD8 then, 50ul whole blood
- 3). Incubate at room temperature, in the dark, for 15 minutes.
- 4). Add 450uL prepared 1X lysing solution II to each tube
- 5). Repeat 3. before acquiring.

NB. For section 2, if this is the first attempt at the Pan-LeucoGating procedure, an extra pair of tubes to set the cytometer , have to be prepared.

### **Acquiring:**

#### **Starting up the FACScan .**

- 1). Turn on the FASCan. This should be done before switching on the computer
- 2). Move the support arm to the right to remove the 'distilled water' tube.
- 3). Check that the sheath fluid tank is full (refill if necessary)
- 4). Check that the waste tank is empty.
- 5). Pressurize the Sheath tank by moving the Pressure switch up.
- 6). Check the sheath filter for trapped air. Gently tap to dislodge any bubbles.
- 7). Open the Optics compartment and while looking at the Flow Cell, turn the Control Dial to 'DRAIN' till the sheath fluid drains from the flow cell, then turn it to 'FILL'  
And watch as the cell fills.  
Repeat this purging until no bubbles are seen in the flow cell as it fills
- 8). Turn the Control Dial to STANDBY mode.
- 9). Switch on the computer.

Open Cell Quest. In the main menu, under the Acquire option, click CONNECT TO CYTOMETER. This allow the user connect the computer to the cytometer and therefore be able to acquire events on loading a sample.

If this is the first attempt at PLG, the extra tubes prepared earlier, are used to create then save your template ie; the graphs, defined gates, and gate statistics for future use.

Plot 1: CD45 (y-axis) Vs SSC

Plot 2: CD4 (y-axis) Vs SSC

Gate 1: drawn off Plot1, defines all leucocytes (CD45+ events).

Gate 2: drawn off Plot 2, defines CD4+ events of all leucocytes (CD45+ events)

Gate 3: defines the lymphocytes of the leucocytes and is drawn  
Off Plot 1.

Proceed to acquire if the plots and gates are drawn.

Ideally, all CD4 tubes, one after the other, for all specimens, are loaded to acquire then, CD8 tubes run. This is merely for convenience. It minimizes time taken gating and re-gating as one switches between CD4 and CD8 tubes.

a). Acquire between 5000 – 10,000 events per sample.

Re-gate if necessary ie; if the cell populations of interest fall outside the pre-defined Regions.

b). Using the statistics (numerations obtained from events acquired in G1, G2 and G3)and the White cell Blood Count (WBC) -which must have been obtained prior to making the run, calculate the absolute CD4 and CD8 values by;

$$\text{Events in Gate 2(G2)/ Events in Gate 1(G1)*WBC}$$

### **Shutting down FACSCan.**

- 1). Turn the Control Dial to STANDBY mode
- 2). In the acquire menu click, DISCONNECT FROM CYTOMETER
- 3). In the File menu, click 'Quit' (quits the Cell Quest program).
- 4). Insert the 'Rinse' tube and turn the dial to RUN. Let be for 5 minutes.
- 5). Remove the rinse tube and insert the 'Distilled Water' tube and run for 5 minutes.
- 6). Leaving the distilled water tube in place, turn the Dial to STANDBY and DEPRESSURISE the system.
- 7). Switch off the machine
- 8). Shut down the computer

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